

Patent Application
Attorney Docket No. 3153.00050/PC10299A

CS weeks of age the piglets were bled and revaccinated with the same vaccine. At approximately 2 months, 3 months, 4 months and 5 months of age all pigs were bled. At approximately 5½ months of age, all spare pigs were removed from the study. At approximately 6 months of age (20 weeks after second vaccination) pigs were bled and 40 pigs were challenged intramuscularly with 2 mL of a virulent culture of *E. rhusiopathiae* (237 mouse LD₅₀, 1.74 x 10⁸ colony-forming units/mL) grown from a culture provided by the National Veterinary Services Laboratory. Animals were monitored for signs of clinical disease and by rectal temperature for 2 days prior to challenge, the day of challenge, and the 7 days following challenge. Any control animal meeting the criterion for elevated rectal temperature (40.9° C) was taken off study and treated with injectable penicillin. Any control animal that had clinical signs of disease, but did not meet the elevated rectal temperature criterion was humanely killed, necropsied, and samples of whole blood, spleen, and liver were cultured for *E. rhusiopathiae*. Any control animal that died was necropsied and samples of spleen and liver were cultured for *E. rhusiopathiae*. Any vaccinated animal meeting the criterion for elevated rectal temperature (40.9° C) and/or clinical signs of disease was taken off study and treated with injectable penicillin. Any vaccinated animal dying following challenge was necropsied and samples of spleen and liver were cultured for *E. rhusiopathiae*. Antibody titers to *E. rhusiopathiae* were determined by ELISA described above and correlation of antibody titers with clinical protection was done.

IN THE CLAIMS:

Rule 12b 23. (New) A method of using a stabilizing agent selected from the group consisting of metal hydroxide, a metal phosphate, an aluminum hydroxide gel, an aluminum phosphate gel, a calcium phosphate gel, a zinc hydroxide/calcium hydroxide gel, and an alum by adding the stabilizing agent to a supernatant of fluid fraction obtained from a *E. rhusiopathiae* culture.

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